(19) Europäisches Patentamt
European Patent Office
Office européen des brevets

(11) EP 0 703 001 B1

(12) EUROPEAN PATENT SPECIFICATION

(45) Date of publication and mention of the grant of the patent:
06.02.2002 Bulletin 2002/06

(21) Application number: **95914536.8**

(22) Date of filing: **06.04.1995**

(51) Int Cl.[7]: **B01J 20/22**, A61M 1/36, B01J 20/32

(86) International application number:
PCT/JP95/00683

(87) International publication number:
WO 95/27559 (19.10.1995 Gazette 1995/45)

(54) ADSORBENT FOR KETOAMINE-CONTAINING PROTEIN

ADSORBENS FUER KETOAMINE ENTHALTENDE PROTEINE

ADSORBANT CONVENANT A UNE PROTEINE CETO-AMINEE

(84) Designated Contracting States:
DE FR GB

(30) Priority: **08.04.1994 JP 7116194**

(43) Date of publication of application:
27.03.1996 Bulletin 1996/13

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EP 0 703 001 B1

Description

TECHNICAL FIELD

[0001] The present invention relates to an adsorbent to remove ketoamine-containing protein, more particularly, it relates to an adsorbent to remove ketoamine-containing protein in body fluid, a process for removing ketoamine-containing protein by using the above-mentioned adsorbent, a process for preventing or treating for diabetic complication by removing ketoamine-containing protein with the adsorbent and an adsorber for ketoamine-containing protein.

BACKGROUND ART

[0002] After a few years have passed since a patient exhibited the onset of diabetes, in most of all the cases, a pathologic change in kidney glomerula appears. Most of the causes of death of diabetics also are due to nephropathy, and recently, the ratio of patients with renal insufficiency which is caused by diabetic nephropathy has been increasing. This is due to angiopathy in kidney glomerula (ref. Diabetes and Renovascular Arteria, PRACTICE, 3, 298 (1986) and Relationship between Diabetes and Blood Vessel, The Bulletin of the Japan Diabetes Society General Meeting, 1 (1988)).

[0003] Although there is present a large amount of sugar in blood of diabetics, compared to normal adults, it is known that glucose which is present in blood bonds to protein and the like without participation of an enzyme. Protein allows to bind to sugar nonenzymatically to form ketoamine-containing protein, then, the ketoamine-containing protein deposits on vessel wall, and, as a result, causes angiopathy and finally allows a serious diabetic complication to proceed. A substance which is obtained by that a reaction for forming ketoamine-containing protein is further proceeded, is known as an Advanced Glycosylation End Products (AGEs). The AGEs is a substance wherein glycosylated proteins are cross-linked to each other, and has been considered as a substance causing diabetic complications (ref. Nonenzymatic Glycosylation and the Pathogenesis of Diabetic Complications; Annals of Internal Medicine, 101, 527(1984) and Advanced Glycosylation End Products in Patients with Diabetic Nephropathy; The New England Journal of Medicine, 325, 836 (1991)).

[0004] Although a treatment for the diabetic complications depends on a vigorous insulin therapy or a depressor therapy currently, once clinically apparent nephropathy progresses, it is difficult to halt the progression of the disease. Alternatively, as to non-insulin dependent diabetes mellitus, there is no effective treatment for the disease now.

[0005] While in the field of food, the fact that sugar adds to protein to give saccharified protein, has been known for a long time, and then a lot of attempts to inhibit glycation have been conducted. For example, in the publications, Japanese Unexamined Patent Publication No. 142114/1987 and Japanese Unexamined Patent Publication No. 156/1990, which were filed with Japanese Patent Office by The Rockefeller University, is described the fact that addition of a composition containing hydrazine or aminoguanidine to desired protein shows an effect inhibiting glycation of the protein. The fact shows an effect that a compound dissolved in aqueous solution inhibits glycation of protein.

[0006] A hydrazide derivative and the like have been employed for carrying ligand in affinity chromatography for a long time (ref. Experiment and Application, Affinity Chromatography: Kodansha (1976)). For example, there is a method wherein a hydrazide derivative which is carried on a carrier is allowed to react with a compound (ligand) containing a carboxyl group in the presence of carbodiimide, and furthermore, to react with aldehyde group of ring-cleaved sugar to fix sugar as a ligand. But the hydrazide derivative-carrying carrier has been used as an adsorbent for affinity. A condition which allows the hydrazide derivative-carrying carrier (adsorbent) to react with aldehyde group needs a higher reaction temperature, a longer reaction time and a larger amount of an aldehyde compound and the like in comparison with a condition being used in extracorporeal circulation (ref. Experiment and Application, Affinity Chromatography: 65 to 79, Kodansha (1976)). This is due to the purpose of the above-mentioned method, that the aldehyde compound and the like are fixed on a carrier. It is extremely unsuitable to allow the synthetic condition of such adsorbent to apply to a condition of adsorption, and therefore, a purpose of adsorption cannot be accomplished at all. It is easily presumed that the reactivity of the carbonyl group of ketoamine is inferior to that of the aldehyde group, and, as a result, a more severe condition such as to raise the reaction temperature or to prolong the reaction time is required. It is impossible, therefore, to adsorb ketoamino group efficiently by employing hydrazide group.

[0007] Insoluble solid supports for the chemical binding of organic compounds are described in US-A-4,419,444, which supports bear at least one active -NH_2.

Further supports are described in WO 90/08172 whereby the support bears a purifying molecule to purify any kind of liquid, for instance liquids like blood or plasma and this purifying molecule is bound to the support through an acylnitride group. Intermediates consisting of supports containing a -$CONHNH_2$ group are mentioned.

[0008] While there has been known a quantitative process for carbonyl group by utilizing a reaction wherein phenylhydrazine reacts with a carbonyl compound to form precipitation, for a long time, and an analytical process for ketoamine by using that process has been studied (ref. J. Biol. Chem., 255 (15), 7218 (1980)). However, the purpose of the above-

mentioned study is solved by allowing ketoamine being present in reaction system to bond to phenylhydrazine at a certain rate (for instance, only 1 %), there is no description about what extent of ketoamine being present in reaction system reacts with phenylhydrazine. To the inventors of the present invention, the extent of the reaction is problem, and furthermore it is often experienced in the field of the art that a reaction carried out in a state of solution can be more efficient than that in solid phase-liquid phase and alternatively a reaction which occurs in a state of solution does not occur in solid phase-liquid phase.

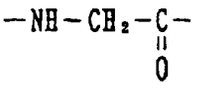
[0009] There is the extremely strong possibility that amine in protein forms ketoamine, a part of the ketoamine has a hydrophobic property, hydrophobic bond occurs, and, as a result, the part of ketoamine is wrapped in the inside of the protein. It seems reasonable to assume that, in case of phenylhydrazine being in a state of solution, it can react with an inside ketoamine of protein, but in case of phenylhydrazine being present in solid phase, only outside part of protein being present in liquid phase can contact with it. It suggests that an invention of an adsorbent wherein a reaction site is ketoamine is not easily achieved. Therefore, a safe and inexpensive adsorbent for treatment in extracorporeal circulation is desired, which can efficiently remove a large amount of ketoamine-containing protein existing in body fluids of patients with diabetic complication.

DISCLOSURE OF THE INVENTION

[0010] The present invention relates to an adsorbent for removing ketoamine-containing protein as defined is claim 1.

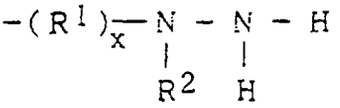
[0011] An adsorber for removing ketoamine-containing protein as defined is claim 6 and a use as defined is claim 7.

[0012] In the present invention, the term "body fluid" means blood, plasma, serum, ascites, lymph, fluid within articular cavity, a constituent of a fraction obtained therefrom and other liquid constituents derived from living body. The term "ketoamine" means the partial structure having the formula:



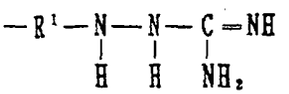
and a substance which is formed by saccharificating protein. The term "ketoamine-containing protein" in the present invention means all the denatured substances which are obtained by binding amino group of protein in living body with reducing sugar nonenzymatically. Those substances include a substance wherein sugar is added to protein to form Schiff base, a substance which forms ketoamine by Amadori rearrangement and a substance which is obtained by the above-mentioned substances being cross-linked to each other (ref. Advanced Glycosylation End Products in Tissue and the Biochemical Basis of Diabetic Complications, The New England Journal of Medicine, 318, 1315 (1988)). The inventors consider that the more protein contains ketoamine, the more the protein has a reaction site and as a result the protein has high pathogenicity.

[0013] In the search for a compound which is effective for adsorbing ketoamine-containing protein, as a result of fixing various compounds to a carrier and examining the effects thereof, it has been found that a compound having a terminal functional group of the formula:



wherein each of R^1 and R^2 is an organic group and X is 0 or 1, was effective for adsorbing ketoamine-containing protein. And in accordance with the above-mentioned inference, the more protein contains ketoamine, the higher the efficiency of adsorption was.

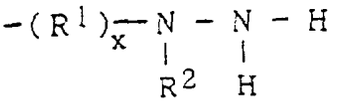
[0014] While each of a compound of the formula:



and a compound of the formula:

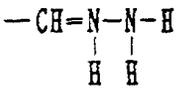
hardly shows ability to adsorb ketoamine-containing protein, and therefore, it was found that terminal -NH_2 is important to adsorb ketoamine-containing protein.

[0015] Alternatively, an introduction of a proper substituent group into the second nitrogen atom from the terminal does not give a great influence, so that R^2 of a group of the formula:

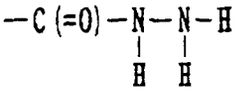


may be a hydrogen atom or an organic group unless R^2 hinders the terminal -NH_2 sterically, and generally a substituent group is hydrogen atom or a substituent group containing 1 to 20 carbon atoms is used.

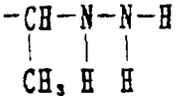
[0016] Furthermore, it is preferable that a bond between the second nitrogen atom from the terminal and the carbon atom neighboring inside of the above nitrogen atom is single bond, and the above neighboring carbon atom has a saturated bond or a bond between the above neighboring carbon atom and the other carbon atom is unsaturated bond. For example, each of hydrazone of the formula:



and hydrazide of the formula:



has a low effect of adsorption, while as shown in a group of the formula:



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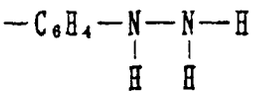
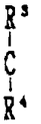


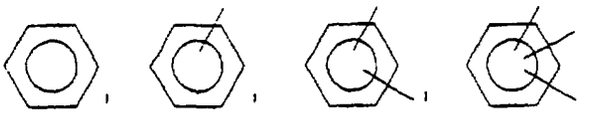
in case a bond between the second nitrogen atom from the terminal and the carbon atom neighboring inside of the above nitrogen atom is single bond, and the above neighboring carbon atom has a saturated bond or a bond between the above neighboring carbon atom and the other carbon atom is unsaturated bond, efficiency of adsorption is high.

[0017] In other words, R^1 has the formula:



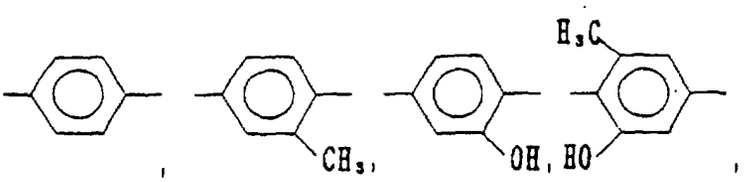
or Ph

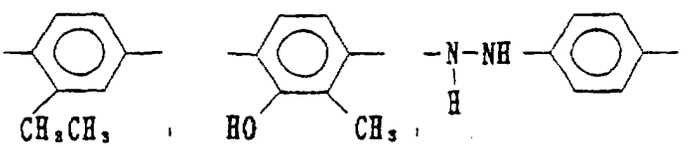
wherein Ph is



or

each of R^3 and R^4 is hydrogen atom, hydroxyl group, amino group or a substituent group having 1 to 20 carbon atoms, is preferable, and R^1 such as CH_2, $CH(CH_3)$, $C(CH_3)_2$, CHOH, $C(OH)CH_3$, $CHCH_2CH_3$, $C(OH)CH_2CH_2CH_3$, $C(NH_2)CH_3$,





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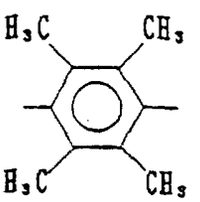


is more preferable and R^1 such as CH_2, $CH(CH_3)$, C_6H_4, NH-NH-C_6H_4 or $C(CH_3)_2$ is particularly preferable.

[0018] R^2 may be any substituent group which binds to nitrogen atom by a single bond, and R^2 having a substituent group of the formula:

-H,

$-C_nH_{2n+1}$,

$-C_nH_{2n}OC_mH_{2m+1}$,

$-C_nH_{2n}OC(=O)C_mH_{2m+1}$,

$-C_nH_{2n}NHC(=O)C_mH_{2m+1}$,

$-C_nH_{2n}C(=O)OC_mH_{2m+1}$,

$-C_nH_{2n}C(=O)NHC_mH_{2m+1}$,

$-C_nH_{2n}SC_mH_{2m+1}$,

$-C_nH_{2n}OH$,

$-C_nH_{2n}NH_2$,

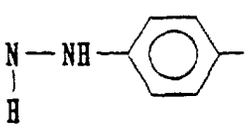
$-C_nH_{2n}NHC_mH_{2m+1}$

or

$-C_nH_{2n}N(C_mH_{2m+1})_2$

wherein each of n and n + m is an integer of 1 to 20, has particularly high ability to adsorb ketoamine-containing protein. R^2 such as H, C_nH_{2n+1}, $C_nH_{2n}OC_mH_{2m+1}$, $C_nH_{2n}OH$, $C_nH_{2n}NH_2$, wherein each of n and n + m is an integer of 1 to 20, is more preferable, and R^2 such as H, CH_3, CH_2CH_3, $C_{16}H_{33}$, CH_2OH, CH_2CH_2OH, CH_2NH_2, $CH_2CH_2NH_2$ or CH_2OCH_3 is particularly preferable.

[0019] Therefore, as a terminal functional group used in the present invention, R^1 such as CH_2, $CH(CH_3)$, $C(CH_3)_2$,



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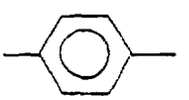
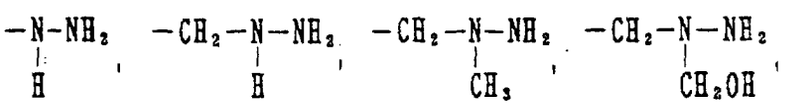
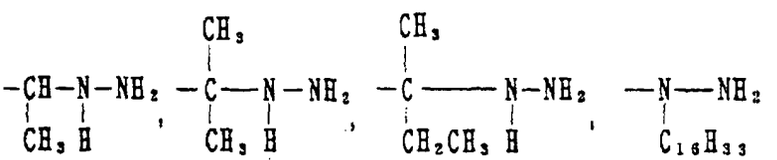


is preferable and as a R^2, a combination of the above-mentioned functional groups is preferable, and R^2 such as





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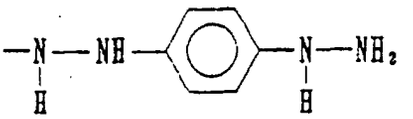
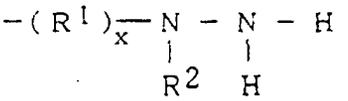


is particularly preferable. The presence of -$CONHNH_2$ is excluded.

[0020] Those compounds may be used alone or a combination of two or more kinds of those compounds may be used.

[0021] At least 10 μmol (per a volume of 1 mℓ of a sedimented water-insoluble carrier) of a compound having a terminal functional group of the formula:



is required to be carried on the carrier, because ability of the compound for adsorption is low, if at most 10 μmol of the compound is carried on the carrier.

[0022] As a water-insoluble carrier used in the present invention, there are an inorganic carrier such as glass beads or silica gel; a synthetic polymer such as crosslinked-polyvinyl alcohol, crosslinked-polyacrylate, crosslinked-polyacryl amide or crosslinked-polystyrene and an organic carrier which comprises of polysaccharide such as crystalline cellulose, crosslinked-cellulose, crosslinked-agarose or crosslinked-dextran, furthermore, a composite carrier obtained from a combination of the above-mentioned compounds such as organic-organic carrier and organic-inorganic carrier. Particularly, a hydrophilic carrier is preferable, because nonspecific adsorption on hydrophilic carrier is relatively poor and good selectivity of adsorption for ketoamine-containing protein can be obtained.

[0023] The term "hydrophilic carrier" used in the present invention means carrier which has at most 60 degrees of contact angle of a compound which constitutes the carrier with water, in case the compound is allowed to form a flat plate. As a representative example of such carriers, there are a carrier comprised of polysaccharide such as cellulose, chitosan, Sepharose or dextran, and a carrier comprised of polyvinyl alcohol, hydrolyzed poly(ethylene-vinyl acetate), polyacrylamide, polyacrylic acid, polymethacrylic acid, poly methyl methacrylate, polyacrylic acid-grafting polyethylene, polyacrylamide-grafting polyethylene or glass.

[0024] Particularly, a carrier wherein OH group is present is excellent in ability for adsorption and selectivity and a carrier comprised of porous cellulose gel is one of the most preferable carriers employed in the present invention, because porous cellulose gel has the following excellent properties:

1. The gel has relatively high mechanical strength and toughness, and as a result of such properties the gel is hardly destroyed or produces fine dividing powder by an operation such as stirring, and when the gel is charged with a column, the gel is not consolidated or clogged up by passing body fluid through the column at high flow rate, furthermore, the structure of pore thereof hardly changes by high-pressure steam sterilization,
2. The gel is comprised of cellulose, so that the gel is hydrophilic, the gel has a large amount of hydroxyl groups which can be employed for bonding ligand and nonspecific adsorption is scarcely caused,
3. The gel has relatively high strength, even if the volume of porosity thereof is enlarged, thus capacity of adsorption thereof which is not inferior to that of soft gel is obtained and
4. Safety of the is higher as compared with that of a synthetic polymer gel and the like. The present invention is not limited to only those carriers. Further, those carriers may be used alone or a mixture of two or more kinds of those carriers may be used.

[0025] A property firstly required for a water-insoluble carrier is that the carrier has many pores having a proper size, namely, that the carrier is porous. A ketoamine-containing protein, which is an object of adsorption by the adsorbent in the present invention, is nonenzymatically glycosylated protein, a molecular weight of the protein is over a wide range and does not determined. In order to adsorb ketoamine-containing protein efficiently, it is preferable that ketoamine-containing protein can penetrate into the pores of a carrier at a certain high probability, but the other protein does not penetrate, if possible.

[0026] Although mercury porosimetry is most frequently employed for measuring a pore size, in the porous water-insoluble carrier used in the present invention, it is mostly impossible to apply the mercury porosimetry. Therefore, it is proper to employ an exclusion limit as a measure of the pore size of the gel. The term "exclusion limit" means the minimum molecular weight of the molecule which cannot penetrate into a pore (i.e. the molecule is excluded) in a gel

permeation chromatograph (ref. Hiroyuki Hatano and Toshihiko Hanai, Experimental High Speed Liquid Chromatography, Kagaku Dojin). Although the exclusion limit has been studied for globular proteins, dextran and polyethylene glycol, in the carrier used in the present invention, exclusion limit measured by employing globular protein is suitably employed.

[0027] As the result of the investigation using carriers having various exclusion limits, it is found that a pore size suitable for adsorbing ketoamine-containing protein has at least 2×10^4 of exclusion limit. Thus, in case of using a carrier having less than 2×10^4 of exclusion limit, the amount of adsorbing and removing ketoamine-containing protein is low and the practicability of the carrier declines. Therefore, the exclusion limit of the carrier used in the present invention is at least 2×10^4.

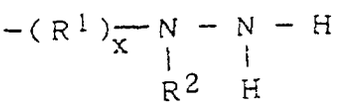
[0028] In case of using a carrier having more than 5×10^6 of exclusion limit, a serious trouble is not caused so long as applying plasma or serum as a body fluid to the carrier, but a macromolecule having no interaction with the ligand tends to close physically the binding site of the ligand, and to decrease the amount of effective ligand.

[0029] Furthermore, in case of applying blood as a body fluid to a carrier, when exclusion limit of the carrier is over 5×10^6, the rate of adhesion of platelet tends to increase, and when the adsorbent of the present invention is applied to DHP (direct blood perfusate) type of hemocatharsis system, the adsorbent cannot always demonstrate enough capability. While, in case of at most 5×10^6 of exclusion limit, a serious trouble is not caused by any usage, thus in order to provide the carrier with flexibility of usage, exclusion limit being at most 5×10^6 is preferable.

[0030] The following is a porous structure of the carrier. In view of the ability of adsorption per unit volume, a structure uniformly having pores at any part of the gel is more preferable than a structure having pores only on the surface of the gel. It is preferred that pore volume of the gel is at least 20 % and that specific surface area of the gel is at least 3 m^2/g. And form of the carrier can be selected from any type of form such as granular, fibrous or hollow type.

[0031] Furthermore, it is suitable for fixing ligand if a functional group which can be used for fixing reaction of ligand is present on the surface of the carrier. As representative examples of those functional groups, there are the hydroxyl group, amino group, aldehyde group, carboxyl group, thiol group, a silanol group, amide group, epoxy group, a halogen, succinylimide group and an acid anhydride group.

[0032] As a carrier used in the present invention, there can be used any one of a hard carrier and a soft carrier, in order to use the carrier for extracorporeal circulation treatment it is important that the gel does not clog up when the carrier is charged in a column and a liquid is passed through the column. Therefore, the gel is required to have sufficient mechanical strength. Thus it is more preferable that the carrier used in the present invention is a hard carrier. The term "hard carrier" used in the present invention means, for instance, in case that a gel is a granulated gel as shown in Reference Example, the carrier wherein the relationship between pressure loss ΔP and flow rate is linear up to 0.3 kg/cm^2 of pressure loss when a cylindrical column is charged with the gel and aqueous fluid is passed through the column. The adsorbent of the present invention is obtained by fixing a compound having a terminal functional group of the formula:



on a porous water-insoluble carrier. As a method of fixation, various methods which are widely known can be employed without particular limitation.

[0033] However, since the adsorbent of the present invention is used for extracorporeal circulation treatment, it is important to suppress desorption and elution of ligand in sterilization or treatment to the utmost from a safety point, thus fixation by using covalent bond is preferable.

[0034] There are various processes for removing ketoamine-containing protein by using the adsorbent of the present invention. The most simple and easy process is a method which comprises taking out blood containing ketoamine-containing protein, storing the blood in a blood bag, mixing the adsorbent of the present invention thereto, removing ketoamine-containing protein, then removing the adsorbent through a filter and returning the blood into the body. The process does not require complicated apparatus, but has a disadvantage that the amount of blood treated at one treatment is little, thereby the treatment needs a long time and the procedure becomes complicated.

[0035] Another process is a process which comprises charging the adsorbent into a column, incorporating it into extracorporeal circulation cycle and carrying out adsorbing for removal on-line. There are processes for treatment such as a process which comprises a direct perfusion of whole blood, and a process which comprises separating plasma from blood and then passing the plasma through a column. The adsorbent of the present invention can be applied to any process of the above-mentioned and is the most suitable for on-line treatment.

[0036] In the extracorporeal circulation cycle described in the present specification, the adsorbent of the present invention can be used alone or in combination with the other extracorporeal circulation treatment system. As an example

of the combination, there is a combination with artificial dialysis cycle, and then, the combination can also be used for hemodialysis therapy.

[0037] An adsorber for removing ketoamine-containing protein of the present invention with the adsorbent for removing ketoamine-containing protein is more specifically explained referring to Fig. 1 which is a schematic cross section of an Example.

[0038] In Fig. 1, 1 represents an inlet for body fluid; 2 represents an outlet for body fluid; 3 represents an adsorbent for removing ketoamine-containing protein of the present invention; 4 and 5 represent a means (filter) for preventing the adsorbent from flowing out, thereby body fluid and a component contained in body fluid can pass but the adsorbent for removing ketoamine-containing protein cannot pass; 6 represents a column; and 7 represents an adsorber for removing ketoamine-containing protein. Shape and material of the container of the above-mentioned adsorber are not particularly limited. As a preferable example, there is a cylindrical column with about 150 to about 400 mℓ of capacity and about 4 to about 10 cm of diameter.

BRIEF DESCRIPTION OF THE DRAWINGS

[0039] Fig. 1 is a schematic cross section of an Example of the adsorber for removing ketoamine-containing protein of the present invention.

[0040] Fig. 2 is a graph showing the result of the relationship between flow rate and pressure loss examined by employing three kinds of gels.

BEST MODE FOR CARRYING OUT THE INVENTION

[0041] The adsorbent of the present invention is more specifically described and explained by means of the following Examples. The present invention is not limited to Examples.

Reference Example

[0042] Each of the cylindrical glass columns equipped with the filters having pore size of 15 μm at both ends thereof (inside diameter: 9 mm, length of the column: 150 mm) was charged uniformly with agarose gel (BIO-GEL A-5m made by Bio-Rad Laboratories, Inc., a particle size: 50 to 100 meshes), vinyl polymer gel (TOYOPEARL HW-65 made by TOSOH Corporation, a particle size: 50 to 100 μm) and cellulose gel (CELLULOFINE GC-700m made by Chisso Corporation, a particle size: 45 to 105 μm), and the relationship between flow rate and pressure loss ΔP was determined by passing water through each of the columns using Peristatic pump. The results are shown in Fig. 2.

[0043] As shown in Fig. 2, it is found that each flow rate in TOYOPEARL HW-65 and CELLULOFINE GC-700m increases in nearly proportion to increase of pressure, but BIO-GEL A-5m is consolidated and the flow rate thereof does not increase in proportion to the increase of pressure. In the present invention, the gel wherein the relationship between pressure loss ΔP and flow rate is a linear relationship up to 0.3 kg/cm^2, as the former, is defined as "hard gel".

EXAMPLE 1

[0044] Into 90 mℓ of GC-700m which is the cellulose porous hard gel (made by: Chisso Corporation, exclusion limit of globular protein: 4×10^5) was added water to give 180 mℓ of total volume. Thereto was added 60 mℓ of 2M sodium hydroxide and the temperature thereof was set at 40°C. Then, thereto was added 21 mℓ of epichlorohydrin and allowed to react with stirring for 1 hour at 40°C. After the reaction was completed, the obtained mixture was fully washed with water to give epoxidated gel.

[0045] Into 90 mℓ of the above epoxidated gel was added a solution wherein 300 mg of hydrazine hydrate was diluted with 20 mℓ of water, and the obtained mixture was left for 20 hours at room temperature and then the mixture was washed with sufficient amount of water to give hydrazine-fixing gel (adsorbent).

[0046] According to a conventional method, the determination of an amount of ligand was carried out employing trinitrobenzenesulfonic acid (TNBS). Into 1 mℓ of each adsorbent was added each of saturated solution of sodium borate (5 g/20 mℓ, 250 μℓ, 210 μmol) and then the amount of ligand was calculated by using the amount of decrease in absorbance determined at 250 nm.

[0047] By reference to the reference (ref. Journal of Biological Chemistry, 255 (15), 7218-7224 (1980)), 400 mg of bovine serum albumin (BSA) (made by SIGMA CHEMICAL COMPANY, FRACTON V) and 7 mg of glyceraldehyde (made by WAKO PURE CHEMICAL INDUSTRIES, LTD.) were dissolved in 6 mℓ of phosphate buffer solution (pH 7.4) (made by DAINIPPON PHARMACEUTICAL CO., LTD.), and the obtained mixture was warmed at 37°C for 20 hours to prepare ketoamine-introducing BSA.

[0048] Into 0.5 mℓ of the adsorbent was added 1.0 mℓ of ketoamine-introducing BSA solution and the obtained

mixture was shaken at 37°C for 2 hours. After shaking, each amount of ketoamine in supernatant solution and in stock solution was determined as an amount of fructosamine by using ROCHE II (made by Nippon Roche K.K.), and a rate of adsorption was calculated. The result is shown in Table 1.

EXAMPLE 2

[0049] In 5 mℓ of phosphate buffer solution (pH 7.4) were dissolved 350 mg of bovine serum albumin and 350 mg of glucose (made by WAKO PURE CHEMICAL INDUSTRIES, LTD.), and the obtained mixture was warmed at 37°C for 20 days to prepare saccharified BSA.

[0050] The procedure of Example 1 was repeated except for employing glycation BSA instead of ketoamine-introducing BSA, and then an amount of fructosamine was determined. The result is shown in Table 1.

EXAMPLE 3

[0051] The procedure of Example 1 was repeated with the adsorbent synthesized in Example 1 except for employing plasma of diabetic instead of ketoamine-introducing BSA, and then an amount of fructosamine was determined. The results is shown in Table 1.

EXMAPLE 4

[0052] The procedure of Example 1 was repeated except for employing methylhydrazine instead of hydrazine hydrate to give methylhydrazine-fixing gel (adsorbent). The experiment of adsorption was performed with that adsorbent, according to the same procedure as described in Example 1. The result is shown in Table 1.

EXAMPLE 5

[0053] According to the reference (ref. J. Org. Chem., 14, 813 (1949)), butylhydrazine was obtained from butylamine. The procedure of Example 1 was repeated except for employing butylhydrazine instead of hydrazine hydrate to give butylhydrazine-fixing gel (adsorbent).

[0054] The experiment of adsorption was performed with that adsorbent, according to the same procedure as described in Example 1. The result is shown in Table 1.

EXAMPLE 6

[0055] The procedure of Example 5 was repeated except for employing cetylamine instead of butylamine to give cetylhydrazine. The procedure of Example 1 was repeated except for employing cetylhydrazine instead of hydrazine hydrate to give cetylhydrazine-fixing gel (adsorbent). The experiment of adsorption of Example 1 was repeated with that adsorbent. The result is shown in Table 1.

EXAMPLE 7

[0056] The procedure of Example 1 was repeated except for employing GCL-200m which is cellulose porous hard gel (made by Chisso Corporation, exclusion limit of globular protein after ligand was fixed: 3×10^6) as a carrier to give hydrazine-fixing gel (adsorbent). The experiment of adsorption of Example 1 was repeated with that adsorbent. The result is shown in Table 1.

EXAMPLE 8

[0057] The procedure of Example 1 was repeated except for employing GC-100m which is cellulose porous hard gel (made by Chisso Corporation, exclusion limit molecular weight of globular protein after ligand was fixed: 3×10^4) as a carrier to give hydrazine-fixing gel (adsorbent). The adsorption experiment of Example 1 was repeated with that adsorbent. The result is shown in Table 1.

EXAMPLE 9

[0058] SEPACOL MINI PP (made by SEIKAGAKU CORPORATION) which is a small polypropylene column was charged with 1 mℓ of methylhydrazine-fixing gel obtained in Example 4, and 6 mℓ of plasma of diabetic was passed through the column. The flow rate of the plasma was controlled with peristalic pump to give about 0.1 mℓ/min of the

flow rate. An amount of fructosamine in effluent was determined by the same procedure of Example 1. The result is shown in Table 1.

EXAMPLE 10

[0059] The procedure of synthesis of epoxidated gel in Example 1 was repeated except for employing 25 mℓ of 2M sodium hydroxide and 8 mℓ of epichlorohydrin to give epoxy-activating gel, and to give hydrazine-fixing gel. The experiment of adsorption of Example 1 was, furthermore, repeated with the ketoamine-introducing BSA solution.

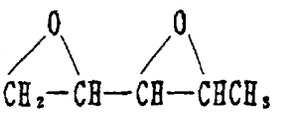
EXAMPLE 11

[0060] The procedure of Example 5 was repeated except for employing p-phenylenediamine instead of butylamine to give p-phenylenedihydrazine.

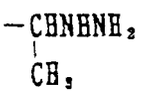
[0061] The procedure of Example 1 was repeated except for employing p-phenylenediamine instead of hydrazine hydrate to give p-phenylenedihydrazine-fixing gel (adsorbent). The experiment of adsorption of Example 1 was repeated with that adsorbent. The result is shown in Table 1.

EXAMPLE 12

[0062] According to the reference (ref. Tetrahedron, 20, 225 (1964)), the procedure of Example 1 was repeated except for employing diglycidylether of the formula:



which was derived from 1, 3-pentadiene, instead of epichlorohydrin to fix a hydrazine derivative having the group of the formula:



and then, to give the hydrazine derivative-immobilizing gel (adsorbent).

[0063] The experiment of adsorption of Example 1 was repeated with that adsorbent. The result is shown in Table 1.

COMPARATIVE EXAMPLE 1

[0064] The procedure of Example 1 was repeated except for employing aminoguanidine instead of hydrazine hydrate to give aminoguanidine-fixing gel. The experiment of adsorption of Example 1 was repeated with that adsorbent. The result is shown in Table 1.

COMPARATIVE EXAMPLE 2

[0065] The procedure of Example 1 was repeated except for employing GCL-90m which was cellulose porous hard gel (made by Chisso Corporation, exclusion limit of globular protein after a ligand was fixed: 1.5×10^3) as a carrier to give hydrazine-fixing gel. The experiment of adsorption of Example 1 was repeated with that adsorbent. The result is shown in Table 1.

COMPARATIVE EXAMPLE 3

[0066] The procedure of Example 4 was repeated except for employing GCL-90m which was cellulose porous hard gel of cellulose (made by Chisso Corporation, exclusion limit of globular protein after a ligand was fixed: 1.5×10^3) as a carrier to give a methylhydrazine-fixing gel. The experiment of adsorption of Example 9 was repeated with that adsorbent. The result is shown in Table 1.

TABLE 1

Ex. No.	Compound employed for fixation (μ mol/mℓ-gel)	Carrier (Exclusion limit)	Adsorbate	Rate of adsorption of ketoamine-containing protein (%)
1	Hydrazine (40)	GC - 700 m (400,000)	Ketoamine-introducing BSA	55
2	Hydrazine (40)	GC - 700 m (400,000)	Saccharified BSA	35
3	Hydrazine (40)	GC - 700 m (400,000)	Plasma of patient	15
4	Methylhydrazine (45)	GC - 700 m (400,000)	Ketoamine-introducing BSA	60
5	Butylhydrazine (15)	GC - 700 m (400,000)	Ketoamine-introducing BSA	50
6	Cetylhydrazine (10)	GC - 700 m (400,000)	Ketoamine-introducing BSA	30
7	Hydrazine (25)	GCL - 2000 m (3,000,000)	Ketoamine-introducing BSA	50
8	Hydrazine (30)	GC - 100 m (30,000)	Ketoamine-introducing BSA	35
9	Methylhydrazine (45)	GC - 700 m (400,000)	Plasma of patient	15
10	Hydrazine (15)	GC - 700 m (400,000)	Ketoamine-introducing BSA	40
11	$NH_2NHC_6H_4NHNH_2$ (25)	GC - 700 m (400,000)	Ketoamine-introducing BSA	30
12	$-CH(CH_3)NHNH_2$ (20)*	GC - 700 m (400,000)	Ketoamine-introducing BSA	35
Com. Ex. 1	Aminoguanidine (35)	GC - 700 m (400,000)	Ketoamine-introducing BSA	20
Com. Ex. 2	Hydrazine (35)	GCL -90 m (15,000)	Ketoamine-introducing BSA	< 5
Com. Ex. 3	Methylhydrazine (20)	GCL- 90 m (15,000)	Plasma of patient	< 5

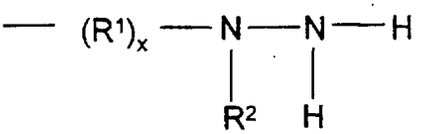
* Structure of fixed-ligand

INDUSTRIAL APPLICABILITY

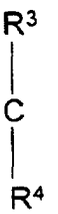
[0067] The present invention can provide an adsorbent which is available at low cost and can adsorb and remove efficiently ketoamine-containing protein in body fluid.

Claims

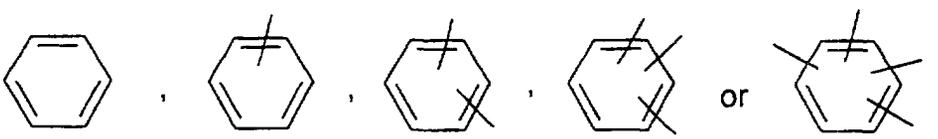
1. An adsorbent for removing ketoamine-containing protein having the partial structure -NH-CH_2-C(=O)- formed by binding an amino-group of protein in a living body with a reducing sugar non-enzymatically,
which comprises carrying a compound having a terminal functional group of the formula:



wherein R^1 has a structure selected from the formula:

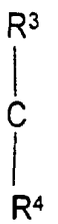


or Ph
wherein Ph is

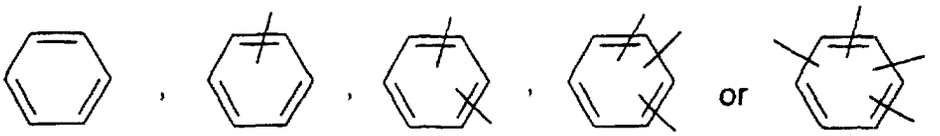


and each of R^3 and R^4 is a hydrogen atom, hydroxyl group, amino group or a univalent organic group, R^2 is a hydrogen atom or an organic group and X is 0 or 1, on a porous water-insoluble carrier having at least 2 x 10^4 of exclusion limit, provided said adsorbent does not contain -$CONHNH_2$.

2. The adsorbent for removing ketoamine-containing protein of claim 1, wherein in said terminal functional group, R^1 is a group of the formula:



or Ph
wherein Ph is



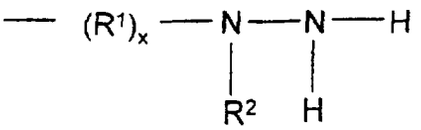
and each of R^3 and R^4 is a hydrogen atom, hydroxyl group, amino group or a substituent group having 1 to 20 carbon atoms; R^2 is a hydrogen atom or a substituent group having I to 20 carbon atoms.

3. The adsorbent for removing ketoamine-containing protein of claim 1 or 2, wherein said porous water-insoluble carrier is hydrophilic.

4. The adsorbent for removing ketoamine-containing protein of any of claims 1 to 3, wherein in said porous water-insoluble carrier is present a terminal functional group of-OH.

5. The adsorbent for removing ketoamine-containing protein of any of claims 1 to 4, wherein the exclusion limit of said water-insoluble carrier is from 2×10^4 to 5×10^6.

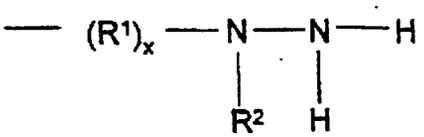
6. An adsorber for removing ketoamine-containing protein having the partial structure -NH-CH_2-C(=O)- formed by binding amino group of protein in living body with reducing sugar non-enzymatically, wherein a container which has an inlet and an outlet for fluid and is equipped with a means preventing the adsorbent for removing ketoamine-containing protein from effusing outside of the container, is charged with the adsorbent for removing ketoamine-containing protein of any of claims 1 to 5. 7.Use of compounds having a terminal functional group of formula:



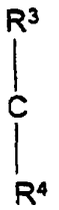
wherein R^1 is an organic group, R^2 is a hydrogen atom or an organic group and X is 0 or 1, which are carried on a porous water-insoluble carrier, for the manufacture of an adsorbent for the removal of ketoamine-containing proteins having the partial structure -NH-CH_2-C(=O)- formed by binding an amino group of a protein in a living body with reducing sugar non-enzymatically.

Patentansprüche

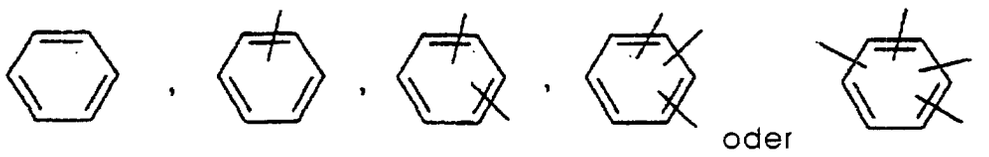
1. Adsorbens für die Entfernung von Ketoamin-enthaltenden Proteinen mit der Partialstruktur -NH-CH_2-C(=O)-, gebildet durch nicht-enzymatisches Verbinden einer Aminogruppe eines Proteins in einem lebenden Körper mit einem reduzierenden Zucker, das umfasst: eine Verbindung mit einer terminalen Gruppe der Formel:



worin R^1 eine Struktur ausgewählt aus der Formel

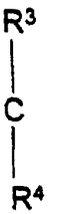


oder Ph ist, worin Ph

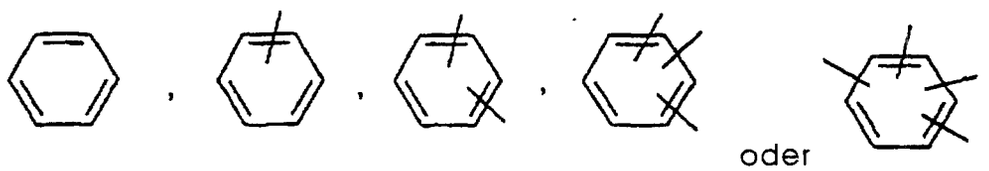


ist, und worin R^3 und R^4 jeweils ein Wasserstoffatom, eine Hydroxylgruppe, eine Aminogruppe oder eine univalente organische Gruppe sind, R^2 ein Wasserstoffatom oder eine organische Gruppe ist und X 0 oder 1 ist, die auf einen porösen wasserunlöslichen Träger mit einer Ausschlußgrenze von mindestens 2 x 10^4 aufgezogen ist, mit der Maßgabe, daß das genannte Adsorbens $-CONHNH_2$ nicht enthält.

2. Adsorbens zur Entfernung eines Ketoamin-enthaltenden Proteins nach Anspruch 1, worin die genannte terminale funktionelle Gruppe R^1 eine Gruppe der Formel



oder Ph ist, worin Ph



ist, und R^3 und R^4 jeweils ein Wasserstoffatom, eine Hydroxylgruppe, eine Aminogruppe oder eine Substituentengruppe mit 1 bis 20 Kohlenstoffatomen sind; und R^2 ein Wasserstoffatom oder eine Substituentengruppe mit 1 bis 20 Kohlenstoffatomen ist.

3. Adsorbens zur Entfernung eines Ketaamin-enihaltenden Proteins nach Anspruch 1 oder 2, worin der genannte wasserunlösliche poröse Träger hydrophil ist.

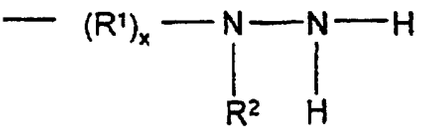
4. Adsorbens zur Entfernung eines Ketoamin-enthaltenden Proteins nach irgend einem der Ansprüche 1 bis 3, worin in dem genannten porösen wassserunlöslichen Träger eine terminale funktionelle -OH-Gruppe vorhanden ist.

5. Adsorbens zur Entfernung eines Ketoamin-enthaltenden Proteins nach irgend einem der Ansprüche 1 bis 4, worin

die Ausschlußgrenze des genannten wasserunlöslichen Trägers von 2 x 10^4 bis 5 x 10^6 ist.

6. Adsorber zur Entfernung eines Ketoamin-enthaltenden Proteins mit der Partialstruktur -NH-CH_2-C(=O)-, gebildet durch nicht-enzymatisches Verbinden einer Aminogruppe eines Proteins in einem lebenden Körper mit einem reduzierenden Zucker, worin ein Behälter, der einen Einlaß und einen Auslaß für eine Flüssigkeit aufweist und der mit Mitteln versehen ist, die verhindern, das das Adsorbens zur Entfernung des Ketoamin-enthaltenden Proteins aus dem Behälter austritt, mit dem Adsorbens zur Entfernung des Ketoamin-enthaltenden Proteins nach irgend einem der Ansprüche 1 bis 5 beladen ist,

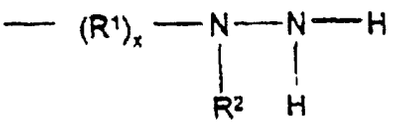
7. Verwendung von Verbindungen mit einer terminalen funktionellen Gruppe der Formel



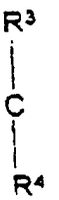
worin R^1 eine organische Gruppe ist, R^2 ein Wasserstoffatom oder eine organische Gruppe ist und X 0 oder 1 ist, die auf einen porösen wasserunlöslichen Träger aufgezogen sind, für die Herstellung eines Adsorbens für die Entfernung eines Ketoamin-enthaltenden Proteins mit der Partialstruktur -NH-CH_2-C(=O)-, gebildet durch nicht-enzymatisches Verbinden einer Aminogruppe eines Proteins in einem lebenden Körper mit einem reduzierenden Zucker.

Revendications

1. Adsorbant pour enlever la protéine céto-aminée ayant la structure partielle -NH-CH_2-C(=O)- formé par la liaison d'un groupe amine de protéine dans un corps vivant avec un sucre de réduction de manière non enzymatique, qui comprend le transport d'un composé ayant un groupe fonctionnel terminal de la formule :

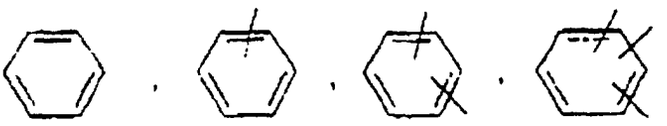


Où R^1 a une structure choisie d'après la formule :



Ou Ph

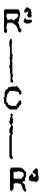
Où Ph est



ou

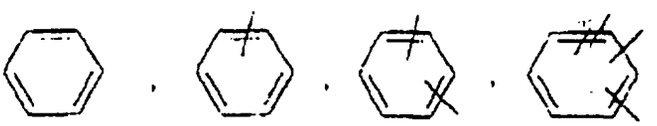
et R^3 et R^4 sont un atome d'hydrogène, groupe d'hydroxyle, groupe amine ou un groupe organique univalent, R^2 est un atome d'hydrogène ou un groupe organique et X est de 0 ou 1, sur un porteur poreux insoluble dans l'eau ayant une limite d'exclusion d' au moins 2 x 10^4, à condition que ledit adsorbant ne contienne pas de -$CONHNH_2$.

2. Adsorbant pour enlever la protéine céto-aminée selon la revendication 1, **caractérisé en ce que** ledit groupe fonctionnel terminal, R^1 est un groupe de la formule :



ou Ph

Où Ph est



ou

et R^3 et R^4 sont des atomes d'hydrogène, groupe d'hydroxyle, groupe amine ou un groupe substituant ayant 1 à 20 atomes de carbone ; R^2 est un atome d'hydrogène ou un groupe substituant ayant 1 à 20 atomes de carbone.

3. Adsorbant pour enlever la protéine céto-aminée selon les revendications 1 ou 2, **caractérisé en ce que** ledit porteur poreux insoluble dans l'eau est hydrophile.

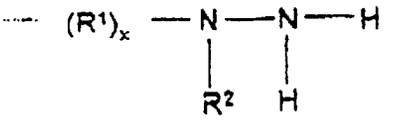
4. Adsorbant pour enlever la protéine céto-aminée selon l'une quelconque des revendications 1 à 3, **caractérisé en ce que** dans ledit porteur poreux insoluble dans l'eau est présent un groupe fonctionnel terminal de -OH.

5. Adsorbant pour enlever la protéine céto-aminée selon l'une quelconque des revendications 1 à 4, **caractérisé en ce que** la limite d'exclusion dudit porteur insoluble dans l'eau est de 2 x 10^4 à 5 x 10^6.

6. Adsorbeur pour enlever la protéine céto-aminée ayant la structure partielle -NH-CH_2-C(=O) - formé en liant le groupe amine de protéine dans un corps vivant avec un sucre de réduction de manière non enzymatique, **carac-**

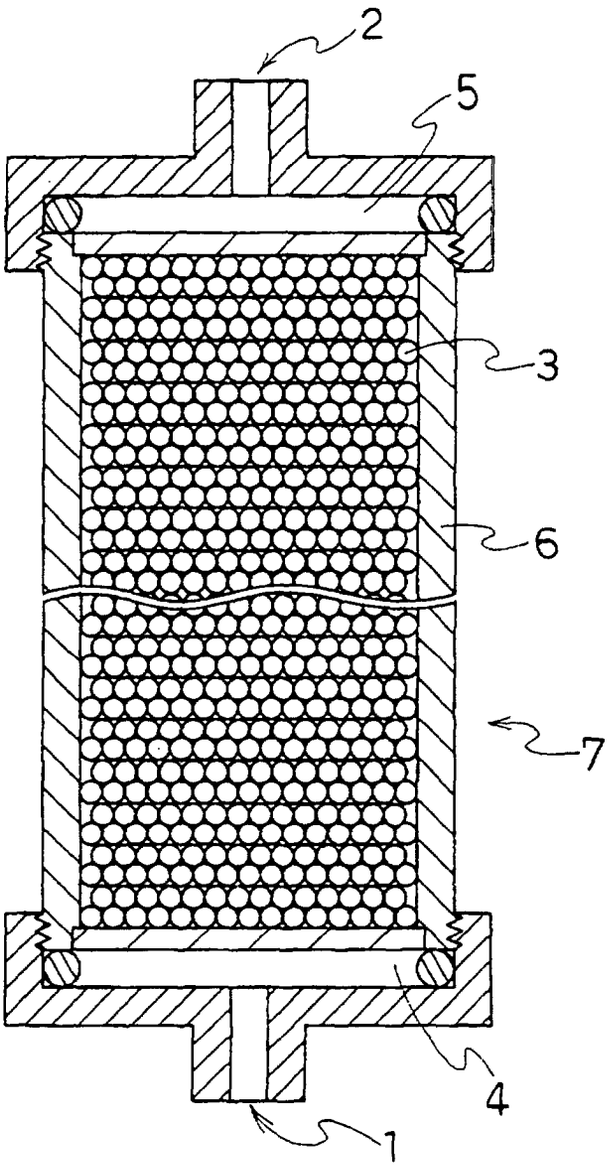
térisé en ce qu'un conteneur qui a une entrée et une sortie pour le fluide et est équipé d'un élément évitant que l'adsorbant qui enlève la protéine céto-aminée ne sorte hors du conteneur, est chargé avec l'adsorbant pour enlever la protéine céto-aminée selon l'une quelconque des revendications 1 à 5.

7. Utilisation de composés ayant un groupe fonctionnel terminal de la formule :



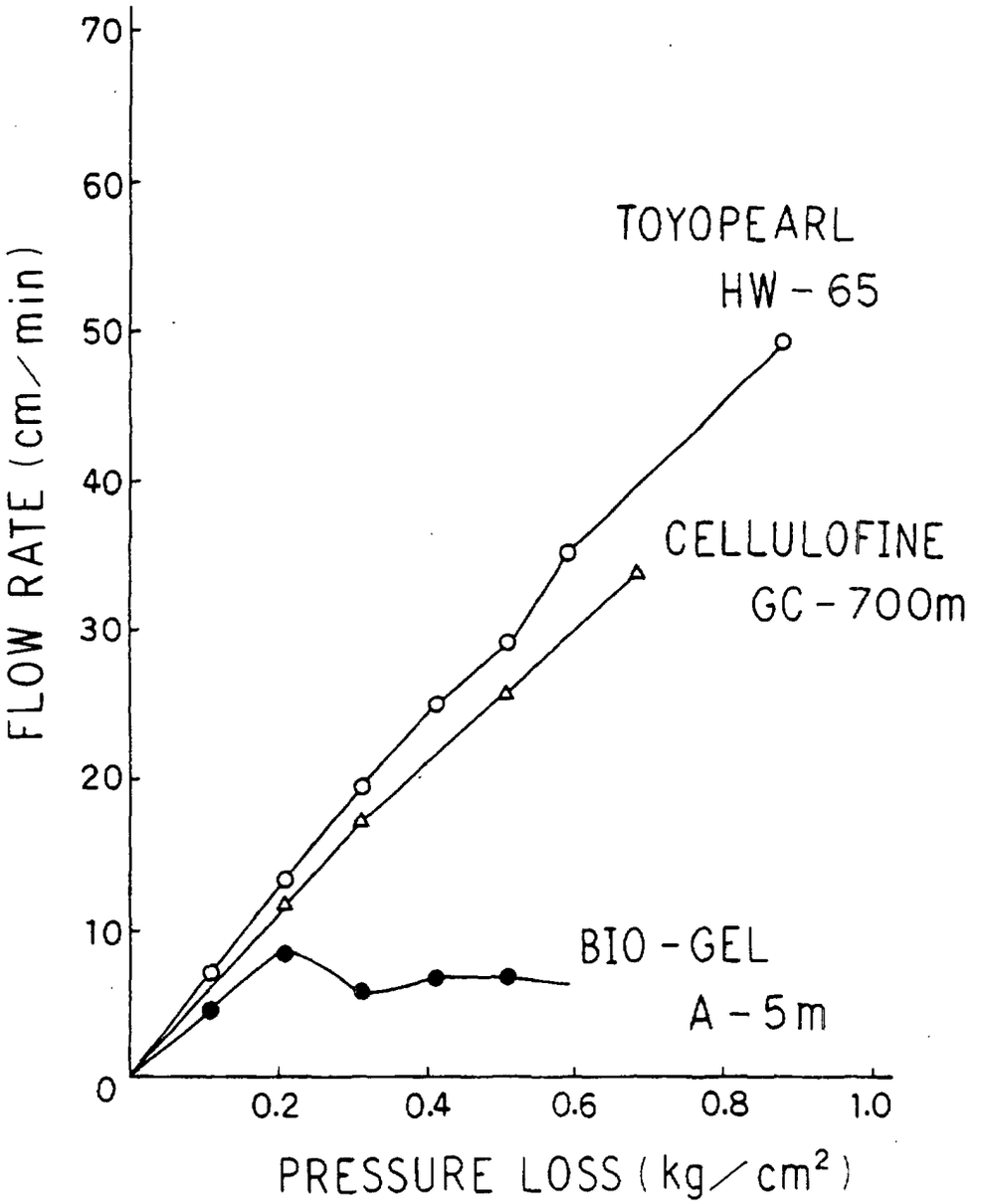
Où R^1 est un groupe organique, R^2 est un atome d'hydrogène ou un groupe organique et X est de 0 ou 1, qui sont réalisés sur un porteur poreux insoluble dans l'eau, pour la fabrication d'un adsorbant permettant d'enlever les protéines céto-aminées ayant la structure partielle -NH-CH_2-C(=O)- formé par la liaison d'un groupe aminé d'une protéine dans un corps vivant avec un sucre de réduction de manière non enzymatique

FIG. 1



1 INLET
2 OUTLET
3 ADSORBENT FOR REMOVING KETOAMINE-CONTAINING PROTEIN
4,5 FILTER
6 COLUMN
7 ADSORBER FOR REMOVING KETOAMINE-CONTAINING PROTEIN

FIG. 2


70
60
50
40
30
20
10
0
FLOW RATE (cm/min)
TOYOPEARL
HW-65
CELLULOFINE
GC-700m
BIO-GEL
A-5m
0.2
0.4
0.6
0.8
1.0
PRESSURE LOSS (kg/cm²)